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B. Webb
8/21/01

DOCKET NO.: 0163-0758-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: :GROUP ART UNIT: 1655

SHINYA KURATA ET AL: :

SERIAL NO.: 09/556,127 :EXAMINER: FREDMAN

FILED: APRIL 20, 2000

FOR: METHOD FOR DETERMINING A CONCENTRATION OF TARGET NUCLEIC ACID MOLECULES, NUCLEIC ACID PROBES FOR THE METHOD, AND METHOD FOR ANALYZING DATA OBTAINED BY THE METHOD

DECLARATION UNDER 37 C.F. R. §1.132

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS

WASHINGTON, D.C. 20231

SIR:

Now comes Shinya KURATA who deposes and states:

1. That I am a graduate of Shimane University at Matsue city and received my Master degree in the year 1993.

2. That I have been employed by Kankyo Engineering Co., Ltd. for 9 years as a researcher in a field of biotechnology.

3. I am an inventor of 09/556,127 and am familiar with the prosecution history thereof.

4. The following experiments were performed by me or under my direct supervision

and control.

5. The following experiments demonstrate that the a probe labeled with BODIPY FL consistent quenching rates when used in a hybridization experiment compared to a similar probe labeled with FITC. The experiments described herein were performed as disclosed in the present application.

6. The BODIPY-modified probe was prepared as follows:

The phosphate group of the deoxycytidylic acid was modified with an amino-linker using a 5' Amino-Modifier C6 kit (Glen Research Inc., USA). An deoxyriboolygonucleotide with a base sequence of 5'ccttcccaca tcgttt3' was synthesized using this modified deoxyribocytidylic acid and other deoxyribonucleosides by using a DNA synthesizer, ABI 394™ (Perkin-Elmer Corp.). After synthesis , protecting groups were released using a 28% ammonia solution. The products were dried and dissolved in a buffer solution (pH, 9.0) of 0.5M $NaHCO_3/Na_2CO_3$ The synthesized deoxyriboolygonucleotide was purified using a NAP-10 column (a product of Pharmacia Inc , Sweden).

A FluoroReporter Kit F-6082 (Molecular Probes, Inc.), which contains BODIPY FL propionic acid succinimidilyl ester and reagents for conjugating the compound to the amine derivative of the above synthesized deoxyriboolygonucleotide, was employed to label the above synthesized deoxyriboolygonucleotide with BODIPY FL at 5'-phosphate. The BODIPY FL-labeled deoxyriboolygonucleotide was purified using the above column and a reverse High Performance Liquid Chromatography method with a SEP-PAK C18 column (6 × 250 mm), a elution solvent (0.5N TEAA 5%CH_3CN) and a gradient solvent B (0.5N TEAA 40%CH_3CN).

7. The FITC labeled probe was prepared as described above for the BODIPY FL-labeled probe.

8. The target nucleic acid was synthesized by using the DNA synthesizer, which

nucleic acid was capable of hybridizing with the above probes and had the same chain length as that of the probes. The target nucleic acid was not labeled.

9. The two aforementioned probes were hybridized to the target nucleic acid as follows:

10. The hybridization was performed at 51°C and the hybridization mixture contained:

Target nucleic acid	320 nM (final concentration)
Nucleic acid probe	80 nM (final concentration)
NaCl	50 nM (final concentration)
$MgCl_2$	1 nM (final concentration)
Tris-HCl buffer (pH 7.2)	100 nM (final concentration)
"MiliQ" purified water	1.6992 mL
final whole volume	2.0000 mL

11. The hybrids formed were measured with excited light at 500 nm for both probes. The fluorescent color was also measured at 520 nm for both brobes.

12. The results of the above-hybridizations yielded a rate of decreased fluorescent intensity of 83% using the BDIPY FL probe, and a fluorescent intensity of 40% using the FITC probe. The rate of fluorescent intensity was measured using the formula:

Rate of decreased fluorescent intensity = 100 - {(fluorescent intensity after hybridization)/(fluorescent intensity before hybridization (before addition of a target nucleic acid in the reaction mixture))} × 100 .

13. The results of this experiment and the experiment presented in Table 4 of the present specification (page 77) clearly demonstrate that the quenching rates of a probe labeled with FITC were variable and may be significantly lower compared to the BODIPY FL labeled probe , which is constant and relatively high.

14. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information are believed to be true.

Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

9. Further deponent saith not.

Shinya Kurata

Sinature Shinya KURATA

August 2, 2001

Date August 2, 2001